

**ORIGINAL RESEARCH**

## Enhancing Sugar Beet Plant Health with Zinc Nanoparticles: A Sustainable Solution for Disease Management

Hadeer Hammad Amin<sup>1</sup>, Abdelanser Badaey Elsayed<sup>2</sup>, Hanafey Farouk Maswada<sup>1</sup>, Nabil Ibrahim Elsheery<sup>1</sup>,

<sup>1</sup>Agriculture Botany Department,  
Faculty of Agriculture, Tanta  
University, Egypt.

<sup>2</sup>Plant Pathology Research Institute,  
Agriculture Research Center  
(ARC), Egypt.

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**ABSTRACT:** Sugar beet (*Beta vulgaris* L.) is susceptible to various diseases, especially powdery mildew, caused by *Erysiphe betae*. Using nanotechnology in agriculture could revolutionize the sector by providing new tools for fast disease diagnosis and disease resistance. This study investigated the potential of Zn nanoparticles in inducing resistance to powdery mildew in sugar beet plants through two experiments. The first experiment assessed the susceptibility of sugar beet cultivars to powdery mildew, with Puma being the most resistant and Top being the most susceptible. The second experiment examined the impact of Zn NPs in inducing resistance to powdery mildew. Zinc-oxide nanoparticles (ZN) and zinc sulfate (ZS) at concentrations of 100, 50 and 10 ppm were used as foliar applications. The results showed that most treatments significantly increased levels of chlorophyll a, b, and total chlorophyll, total soluble sugars, endogenous H<sub>2</sub>O<sub>2</sub>, and activity of peroxidase (POD) and polyphenol oxidase (PPO), while reducing the severity of powdery mildew disease, lipid peroxidation (MDA), phenolics concentrations and catalase activity, especially Zn at concentrations of 100 and 50 ppm compared to infected control. The physiological role of Zn NPs in inducing resistance against powdery mildew disease is attributed to the production and accumulation of reactive oxygen species (ROS) and oxidative reactions of phenolic compounds catalyzed by PPO and/or POD. Our results suggested that ZnO nanoparticles at 100 and 50 ppm can be used as a foliar spray to reduce the harmful impacts of biotic stress caused by *E. betae* in sugar beet plants by inducing resistance to the pathogen.

**KEYWORDS:** Sugar beet, powdery mildew, nano zinc-oxide, zinc sulfate

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### 1. Introduction

Sugar beet (*Beta vulgaris* L.) is a relatively new crop grown in temperate regions and widely spread in the twentieth century. It is grown in 50 countries and is an exemplary crop for sugar production in Egypt (James, 2004). The crop's cultivation area increases annually to meet the demands of the growing population, with a total production of 14.19

million tons in Egypt alone (FAO, 2021). However, powdery mildew caused by *Erysiphe betae* is one of the significant plant pathogens that attack sugar beet leaves, leading to a major reduction in root yield, with a 22% decrease in root yield and a 13% reduction in sucrose content (Kontradowitz and Verreet, 2010). Chemical control is both economically costly and harmful to the

environment, and fungicides can lead to the development of resistant strains of pathogens (Abdel-Monaim and Abo-Elyousr, 2012; El-Fahar and Abou El-Magd, 2008). Therefore, developing eco-friendly methods and alternative economic models for managing plant diseases is crucial. Induced resistance has been extensively studied as a promising non-traditional and eco-friendly approach for providing systemic protection against powdery mildew (Biswas et al., 2012). Agricultural scientists have investigated the potential of nanoparticles for controlling phytopathogens, inspired by the documented antimicrobial properties of various nanomaterials against pathogens affecting humans and livestock, as reported by Biswas et al. (2012). The main advantage of using nanoparticulate fungicides is that they can provide the same or better results than bulk-salt formulations at lower application dosages, reducing ecotoxicity and phyto-toxicity issues caused by the release of metallic cations (Eisa et al., 2006).

However, the development of eco-friendly and sustainable methods of managing plant diseases remains a challenge. With the growing demand for sugar beet, it is essential to ensure the sustainability of its cultivation and production EL-Sayed et al. (2014). The use of fungicides and pesticides is not economically viable and environmentally sustainable (Kontradowitz and Verreet, 2010). Induced resistance and nanotechnology offer promising alternatives to traditional methods of disease management (Ishii et al., 2001; Reuveni and Reuveni, 2000).

Sugar beet production is essential for the economies of many countries. In 2021, Egypt produced a total of 14.83 million tons, which

was a decrease compared to 15.34 million tons in 2020 (FAO 2021). Powdery mildew disease caused by *Erysiphe betae* significantly affects sugar beet production, leading to a major reduction in root yield and sucrose content (Kalia et al., 2020; Kaur et al., 2020; Munir et al., 2019). Chemical control using fungicides is both costly and harmful to the environment, leading to the development of resistant strains of pathogens (Sanchez-Lopez et al., 2020; Singh et al., 2019; Diez-Pascual, 2018). Therefore, eco-friendly methods of disease management are required.

Induced resistance is a promising non-traditional and eco-friendly approach for providing systemic protection against powdery mildew (Elmer and White, 2018). Agricultural scientists have explored the use of nanoparticles to control phytopathogens, following evidence of the cidal characteristics of several types of nanomaterials against human/livestock pathogens (Raliya and Tarafdar, 2013; Choudhury et al., 2010). The use of nanoparticulate fungicides offers a significant advantage, providing the same or better results than bulk-salt formulations at lower application dosages, which reduces ecotoxicity and phyto-toxicity issues caused by the release of metallic cations (Tirani et al., 2019; Abd-Elsalam, 2012; Velusamy (2013).

In the present study, we explored the use of nanotechnology in the management of powdery mildew disease in sugar beet. Furthermore, we discussed the effectiveness of nanoparticles in controlling powdery mildew disease in sugar beet and compare the results with traditional fungicides. In addition, we also discussed the possible benefits of using nanotechnology in disease management and the potential risks associated with its use.

By studying the effectiveness of nanoparticulate fungicides, this research paper aims to contribute to the development of eco-friendly and sustainable methods of managing plant diseases, particularly in sugar beet production, to ensure the sustainability of its cultivation and production.

## 2. Materials and methods

The current experiment was conducted during the 2018-2019 and 2019-2020 growing seasons. Experiments were carried out at the Laboratory and the greenhouse facilities at Gemmeiza Agricultural Research Centre (ARC) and the Faculty of Agriculture, Tanta University.

### 2.1. Evaluation of Sugar Beet Cultivars for Powdery Mildew Resistance:

Eight sugar beet (*Beta vulgaris* L.) cultivars, namely Top, Toro, Carola, Lola, Puma, Gazell, Heba, and Oscar poly., were obtained from the Maize and Sugar Crops Disease Research Department Plant Pathology Research Institute Agriculture Research Center (ARC), Giza, Egypt. The first experiment was conducted to evaluate the resistance/sensitivity of these cultivars to powdery mildew disease, which is caused by *Erysiphe beta**e*.

### 2.2. Greenhouse Conditions and Artificial Inoculation:

The greenhouse experiment was performed using artificial inoculation with *Erysiphe beta**e*, the causal agent of powdery mildew in sugar beet. The seeds of the tested cultivars were sown in micro plots. The experiment was designed in a completely randomized block with three replicates. The inoculation method for powdery mildew was carried out as follows: sugar beet cultivars, which were 8

weeks old, were sprayed with spores of *Erysiphe beta**e* by gently shaking the conidia from the leaves of infected plants on the top of the tested plant leaves (El-Zahaby et al., 1995). The plants were then irrigated and incubated under controlled conditions until disease development.

### 2.3. Disease assessment

The severity of the disease [DS (%)] was evaluated for each cultivar 14, 35, and 49 days' post artificial inoculation based on the powdery mildew scale described by Descalzo et al. (1990) with a slight modification by El-Habbak (2003), which is presented in Table 1.

Table 1. Disease assessment scale.

Scale	Number of colonies per leaf
0	no mildew
1	1-25 colonies/leaf
2	26-50 colonies/leaf
3	51-75 colonies/leaf
4	76-100 colonies/leaf
5	more than 100 colonies/leaf

The severity of the disease [DS (%)] was evaluated for each cultivar 14, 35, and 49 days post artificial inoculation based on the powdery mildew scale described by Descalzo et al. (1990) with a slight modification by El-Habbak (2003), which is presented in Table 1.

The disease severity percentage [DS (%)] was calculated utilizing the equation suggested by Townsend and Heuberger (1943) as follows:

$$\text{Disease severity (\%)} = \frac{\sum(\text{colony count}) \times 100}{\text{number of leaves}}$$

The efficiency of each treatment in the reduction of DS (%) compared to the control was calculated utilizing following equation:

$$\text{Efficiency (\%)} = (\text{control} - \text{treatment}) / \text{control} \times 100$$

#### **2.4. Effect of the chemical inducers to powdery mildew on sugar beet and its yield and quality**

Based on the findings of our initial experiment, we have developed a second experiment to further explore and expand upon our results.

#### **2.5 Host plant**

The most susceptible variety in sugar beet to powdery mildew disease was (Top) variety. The fungicide: Opera 18.3% SE (pyraclostrobin / Epoxiconazole), BASF Company for Chemicals and Pesticides was used at the recommended dose in inducing resistance of powdery mildew as a reference in this study.

#### **2.6 Characterization of nano-zinc and its readiness for use**

The experiment was performed in a split plot design with three replicates where (nano zinc-oxide ( $n\text{ZnO}$ ),  $\text{ZnSO}_4$  and (Opera as fungicide) were applied in the main plots whereas their different concentrations of application (10, 50 and 100  $\text{mg l}^{-1}$ ) and the fungicide were schemed in the sub-plots (Elsheery, 2020).

Preparation of substances used in the study:

#### **2.6. Experimental design and growth conditions**

The experiment was conducted using a randomly complete block design (RCBD) with three replicated plots for each treatment. There were eight treatments with three replicates for each treatment distributed in a total of 24 plots. Each plot measured 4 x 3 m<sup>2</sup>.

Sugar beet seeds of the Top cultivar were planted with a spacing of 60 cm between plants inter-row and 90 cm between plant rows. Three seeds were planted per hill and the plants were grown under normal greenhouse conditions with a temperature range of 25-30 °C and relative humidity of 75-80%. After 15 days, the seedlings were thinned to one per hill. Artificial inoculation was performed as previously described (El-Zahaby et al., 1995). Root weight in kilograms was recorded, as well as quality features such as total soluble solids (T.S.S) percentage, which was measured in fresh roots using a hand refractometer following the method described by McGinnis (1982). Sucrose percentage was determined using a succharometer in accordance with the Sambucetti et al. (1996). Purity percent was calculated by dividing the sucrose by T.S.S. Root weight in kilograms was also recorded.

#### **2.7. Chlorophyll concentration**

The concentration of chlorophyll was determined as follows, based on the method described by Deer et al. in 1998. Fresh leaves (0.1 g) were cut into small fragments (1 mm x 1 mm) and soaked in 20 ml of methanol (96%) for 24 hours at 4°C. The resulting mixture was then filtered through Whatman 47 mm GF/C filter paper. The absorbance of each filtrate was measured against a blank of 96% methanol at wavelengths of 666 and 653 nm for chlorophyll a and b, respectively. The results were expressed as  $\text{mg g}^{-1}$  fresh weight (FW) and were calculated using the following formulas:

$$\text{Chl a} = [(15.65 \times A_{666} - 7.34 \times A_{653}) \times (V/W)] / 1000$$

$$\text{Chl b} = [(27.05 \times A_{653} - 11.21 \times A_{666}) \times (V/W)] / 1000$$

Where V is the volume of methanol extract (ml), and W is the weight of plant leaf sample (g).

## 2.8 Statistical analysis

Data collected were subjected to a contrast analysis in accordance with (Steel and Torrie, 1960). To compare means, multi range Duncan tests were enforced too (Duncan, 1955). Figures were drawn by using excel 2016.

## 3. Results

### 3.1 Evaluation the resistance of sugar beet cultivars to powdery mildew

The results demonstrate that sugar beet cultivars exhibited varying responses to *E. betae* infection, with these differences becoming more pronounced after 49 days post inoculation (dpi) (Figure. 1). The cultivar Puma demonstrated the highest level of resistance, with a DS of 20.78%, while the Top cultivar exhibited the greatest susceptibility to powdery mildew disease, recording a maximum DS of 87.08%. These findings indicate that the Top cultivar is particularly vulnerable to powdery mildew disease. We found that Puma cultivar was the most resistance in the tested cultivars while Top cultivar was the most sensitive one (Figure 1).

### 3.2 Effect of the chemical inducers to powdery mildew on sugar beet and it's yield and quality

The greenhouse experiment was conducted based on the best exogenous treatments obtained from the first experiment which showed that Top cultivar (high susceptible to PMD) was selected in the second experiment to investigate the potential role of foliar spray

with nano-ZnO (NZ) at different concentrations (10, 50 and 100 mg l<sup>-1</sup>) to induce its resistance to PMD compared with zinc sulfate (ZS) at the same concentrations (10, 50 and 100 mg l<sup>-1</sup>), fungicide (Opera) at recommended dose, and control (untreated plants) under controlled greenhouse conditions.

### 3.3 Effect of chemical inducers on the disease severity (%)

After artificial inoculation with *Erysiphe betae*, the disease severity [DS (%)] was recorded in each treatment at 14, 28, 42, 56 and 70 days post inoculation (dpi). At 14 dpi, the only significant treatment did not show significant suppression compared with control (Figure. 2 and 3). Fungicide and nano-ZnO at 100 mg l<sup>-1</sup> (NZ<sub>3</sub>) were the only efficient treatments for reducing PMD at 28 dpi. All foliar applications with the investigated compounds on inducing the systemic resistance of plants against *E. betae* greatly reduced disease severity of powdery mildew at 42, 56 and 70 dpi. Results indicated that, all exogenous treatments particularly fungicide Opera, nano-ZnO at 100 and 50 mg l<sup>-1</sup>, zinc sulfate at 100 mg l<sup>-1</sup> could be used as inducers for reducing the disease severity [DS (%)] of powdery mildew in susceptible cultivar (Top) of sugarbeet plants.

### 3.4 Photosynthetic pigments

The concentration of (Chl a) significantly increased over control due to all exogenous treatments whether before or after inoculation (Table 2). It is also noticed that Chl a concentration significantly decreased after inoculation (AI) compared with before inoculation (BI). In the same manner, Chl b concentration was significantly increased due

to chemical inducers (nano-ZnO, Zn SO<sub>4</sub> and fungicide) compared with control (Table 3).

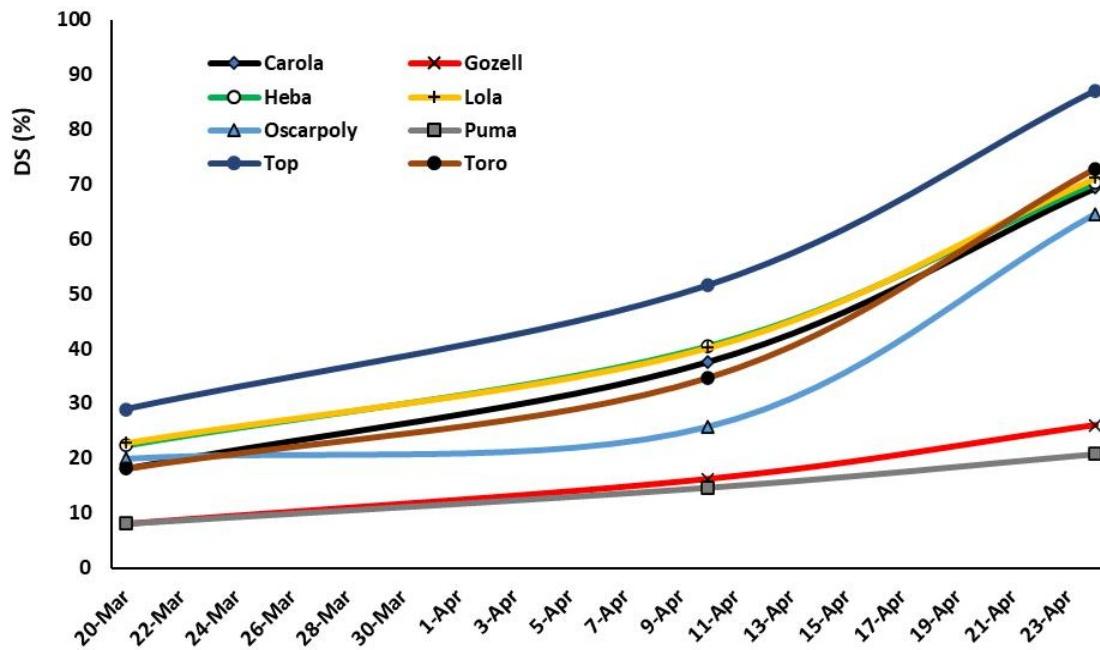


Figure 1. Susceptibility of different sugar beet varieties to artificial inoculation with *E. betae* the fungus of causal powdery mildew under greenhouse conditions.

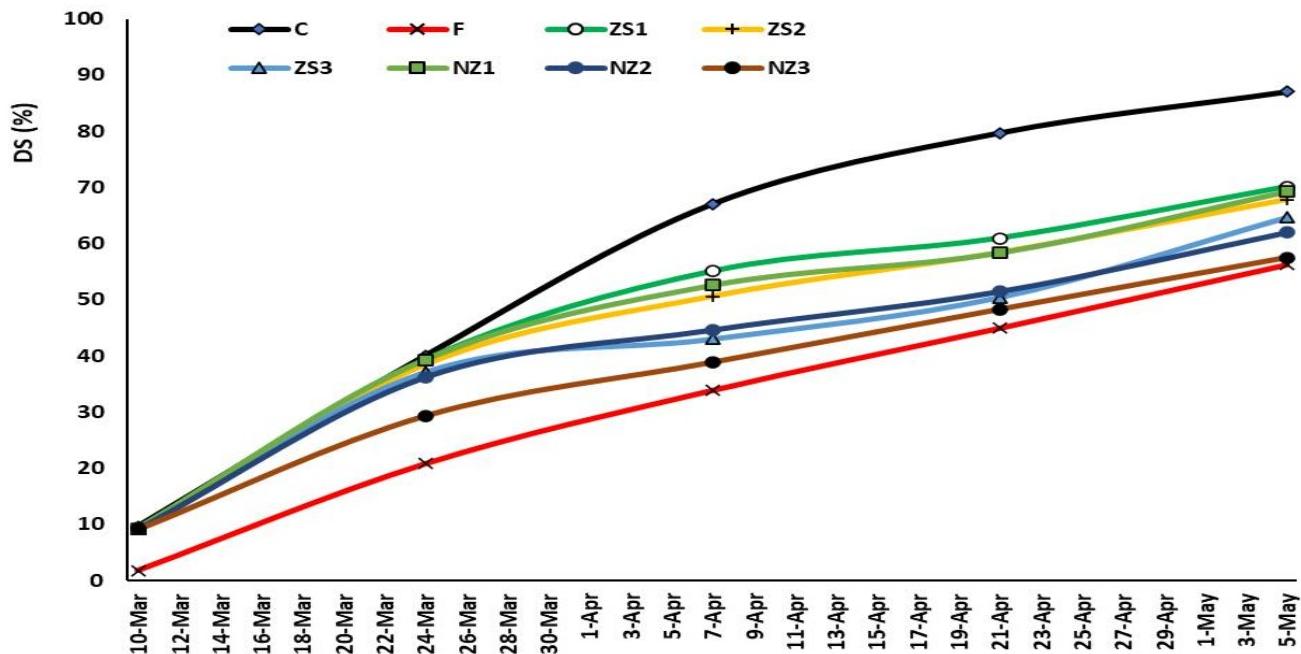


Figure 2. Effect of chemical inducers on the disease severity (%) of sugar beet (Top cultivar) at different periods (14, 28, 42, 56 and 70 days post artificial inoculation) under greenhouse.

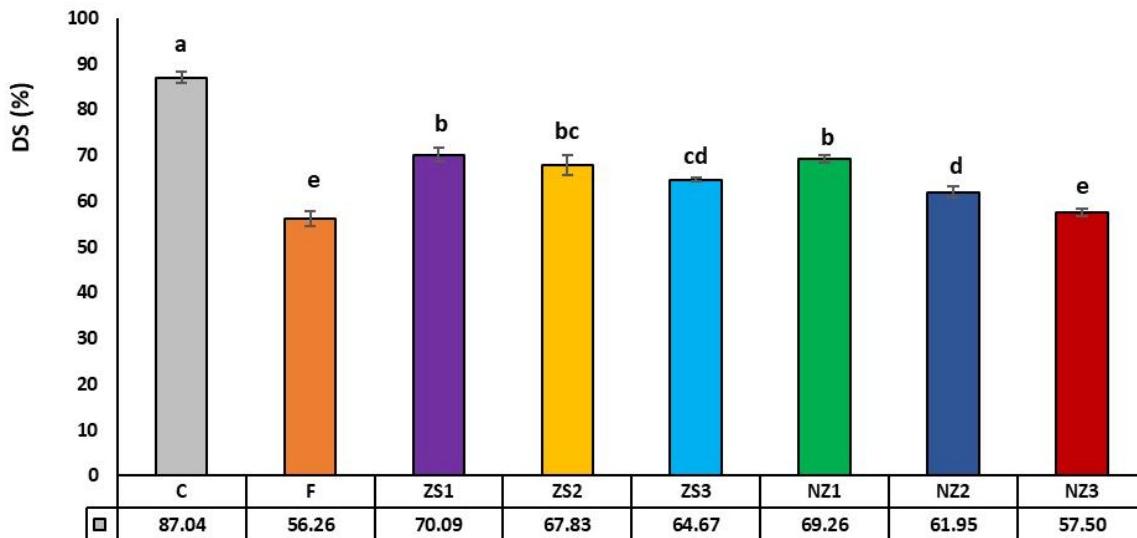


Figure 3. Effect of chemical inducers on the disease severity (%) of sugar beet (Top cultivar) at 70 days post artificial inoculation under greenhouse conditions. Bars are mean  $\pm$  standard error of three replicates of each treatment. Means values within each bar followed by different letters are significantly different ( $P \leq 0.05$ ), according to Duncan's multiple range test. Note: C, control; F, fungicide Opera; ZS<sub>1</sub>, zinc sulfate at  $10 \text{ mg l}^{-1}$ ; ZS<sub>2</sub>, zinc sulfate at  $50 \text{ mg l}^{-1}$ ; ZS<sub>3</sub>, zinc sulfate at  $100 \text{ mg l}^{-1}$ ; NZ<sub>1</sub>, nano-zinc oxide at  $10 \text{ mg l}^{-1}$ ; NZ<sub>2</sub>, nano-zinc oxide at  $50 \text{ mg l}^{-1}$ ; NZ<sub>3</sub>, nano-zinc oxide at  $100 \text{ mg l}^{-1}$ .

Table 2. Effect of chemical inducers on the concentration of chlorophyll a in sugar beet leaves before (BI)

Treatments	BI	AI	Mean
C	$0.610 \pm 0.005\text{g}$	$0.538 \pm 0.005\text{g}$	$0.574 \pm 0.001\text{ h}$
F	$0.707 \pm 0.001\text{e}$	$0.661 \pm 0.016\text{e}$	$0.684 \pm 0.009\text{ f}$
ZS <sub>1</sub>	$0.668 \pm 0.008\text{f}$	$0.599 \pm 0.003\text{f}$	$0.633 \pm 0.004\text{ g}$
ZS <sub>2</sub>	$0.737 \pm 0.020\text{de}$	$0.693 \pm 0.009\text{d}$	$0.715 \pm 0.011\text{ e}$
ZS <sub>3</sub>	$0.807 \pm 0.009\text{c}$	$0.749 \pm 0.009\text{c}$	$0.778 \pm 0.004\text{ c}$
NZ <sub>1</sub>	$0.848 \pm 0.013\text{b}$	$0.793 \pm 0.008\text{b}$	$0.820 \pm 0.005\text{ b}$
NZ <sub>2</sub>	$0.905 \pm 0.015\text{a}$	$0.843 \pm 0.007\text{a}$	$0.874 \pm 0.007\text{ a}$
NZ <sub>3</sub>	$0.769 \pm 0.005\text{d}$	$0.714 \pm 0.007\text{d}$	$0.742 \pm 0.003\text{ d}$
Mean	$0.756 \pm 0.016\text{A}$	$0.699 \pm 0.017\text{B}$	

and after inoculation (AI) with *Erysiphe betae*.

Note: C, control; F, fungicide Opera; ZS<sub>1</sub>, zinc sulfate at  $10 \text{ mg l}^{-1}$ ; ZS<sub>2</sub>, zinc sulfate at  $50 \text{ mg l}^{-1}$ ; ZS<sub>3</sub>, zinc sulfate at  $100 \text{ mg l}^{-1}$ ; NZ<sub>1</sub>, nano-zinc oxide at  $10 \text{ mg l}^{-1}$ ; NZ<sub>2</sub>, nano-zinc oxide at  $50 \text{ mg l}^{-1}$ ; NZ<sub>3</sub>, nano-zinc oxide at  $100 \text{ mg l}^{-1}$ .

Table 3. Effect of chemical inducers on the concentration of chlorophyll b in sugar beet leaves before (BI) and after inoculation (AI) with *Erysiphe betae*.

Treatments	BI	AI	Mean
C	0.232 ± 0.004 g	0.229 ± 0.001 f	0.231 ± 0.002 g
F	0.267 ± 0.001 e	0.251 ± 0.003 e	0.259 ± 0.002 e
ZS <sub>1</sub>	0.252 ± 0.005 f	0.229 ± 0.004 f	0.240 ± 0.003 f
ZS <sub>2</sub>	0.271 ± 0.008 e	0.259 ± 0.003 de	0.265 ± 0.003 e
ZS <sub>3</sub>	0.294 ± 0.002 c	0.280 ± 0.010 c	0.287 ± 0.004 c
NZ <sub>1</sub>	0.309 ± 0.002 b	0.294 ± 0.005 b	0.301 ± 0.004 b
NZ <sub>2</sub>	0.324 ± 0.003 a	0.307 ± 0.005 a	0.316 ± 0.002 a
NZ <sub>3</sub>	0.282 ± 0.004 d	0.265 ± 0.005 d	0.274 ± 0.004 d
Mean	0.279 ± 0.005 A	0.264 ± 0.005 B	

Note: C, control; F, fungicide Opera; ZS<sub>1</sub>, zinc sulfate at 10 mg l<sup>-1</sup>; ZS<sub>2</sub>, zinc sulfate at 50 mg l<sup>-1</sup>; ZS<sub>3</sub>, zinc sulfate at 100 mg l<sup>-1</sup>; NZ<sub>1</sub>, nano-zinc oxide at 10 mg l<sup>-1</sup>; NZ<sub>2</sub>, nano-zinc oxide at 50 mg l<sup>-1</sup>; NZ<sub>3</sub>, nano-zinc oxide at 100 mg l<sup>-1</sup>.

Table 4. Effect of chemical inducers on chlorophyll a/b ratio in sugar beet leaves before (BI) and after inoculation (AI) with *Erysiphe betae*.

Treatments	BI	AI	Mean
C	2.63 ± 0.030 b	2.34 ± 0.023 b	2.49 ± 0.024 c
F	2.64 ± 0.013 b	2.63 ± 0.038 a	2.64 ± 0.017 b
ZS <sub>1</sub>	2.65 ± 0.058 b	2.62 ± 0.049 a	2.64 ± 0.052 b
ZS <sub>2</sub>	2.72 ± 0.028 ab	2.67 ± 0.021 a	2.70 ± 0.013 ab
ZS <sub>3</sub>	2.74 ± 0.033 ab	2.68 ± 0.057 a	2.71 ± 0.039 ab
NZ <sub>1</sub>	2.75 ± 0.063 ab	2.70 ± 0.038 a	2.73 ± 0.045 ab
NZ <sub>2</sub>	2.79 ± 0.043 a	2.74 ± 0.024 a	2.77 ± 0.026 a
NZ <sub>3</sub>	2.72 ± 0.059 ab	2.70 ± 0.034 a	2.71 ± 0.027 ab
Mean	2.71 ± 0.017 A	2.64 ± 0.024 B	

Note: C, control; F, fungicide Opera; ZS<sub>1</sub>, zinc sulfate at 10 mg l<sup>-1</sup>; ZS<sub>2</sub>, zinc sulfate at 50 mg l<sup>-1</sup>; ZS<sub>3</sub>, zinc sulfate at 100 mg l<sup>-1</sup>; NZ<sub>1</sub>, nano-zinc oxide at 10 mg l<sup>-1</sup>; NZ<sub>2</sub>, nano-zinc oxide at 50 mg l<sup>-1</sup>; NZ<sub>3</sub>, nano-zinc oxide at 100 mg l<sup>-1</sup>.

The highest Chl b increase (39.7 and 33.9 %) over control was obtained in NZ<sub>2</sub>-treated plants BI and AI, respectively. Moreover, Chl-b concentration was decreased AI compared with BI. It is worthy to denote that, the effect of infection with PMD on the decrease in Chl-b was lower compared with its effect on Chl-a.

Chl a/b ratio was increased in treated plants compared with control whether BI or AI (Table 4). All treatments, particularly NZ<sub>2</sub>, significantly increased Chl a/b ratio. In addition, the inoculation with *E. betae* significantly decreased Chl a/b ratio, where the Chl a/b ratio was lower in AI than that in BI.

These results indicated the potential role of exogenous treatments as chemical inducers for enhancing chlorophyll content in infested sugar beet plants.

### **3.5. Effect of chemical inducers on yield and quality of sugar beet plants**

Foliar spray with nano-ZnO was more effective increasing sugar beet yield than obtained by plants sprayed with zinc sulfate. The average increase in root yield over control (47.4 and 19.3 %) was achieved by nano-ZnO and ZnSO<sub>4</sub>, respectively (Fig.4). On the other hand, compared with control, no significant increase in total soluble solids (TSS) in sugar beet plants treated with all tested treatments except NZ<sub>2</sub> (nano-ZnO at 50 mg l<sup>-1</sup>) (Figure.5).

The maximum increase in sucrose content (94.2 %) was recorded in NZ<sub>2</sub>-treated plants. The increase in sucrose content over control achieved by NZ<sub>1</sub>, ZS<sub>3</sub>, NZ<sub>3</sub>, ZS<sub>2</sub>, Opera and SZ<sub>1</sub> was 73.7, 47.0, 34.6, 29.5, 21.9 and

12.6 %, respectively (Figure. 7 and 8), all exogenous treatments significantly ( $P<0.05$ ) improved sucrose purity compared with control.

## **4. Discussion**

### **4.1 Evaluation the resistance of sugar beet cultivars to powdery mildew**

The results of our first experiment revealed significant variation in disease severity among the cultivars. The combined data over the season showed that Puma and Gazell cultivars were highly resistant to the powdery mildew disease pathogen compared to Oscar poly, which was moderately resistant. Meanwhile, Carola, Heba, Lola, and Toro cultivars were moderately susceptible, while (Top) was the most susceptible to powdery mildew disease caused by *E. betae*. These findings are in line with a report by El-sayed (2015), which stated that Puma, Oscar poly, and Carola were highly resistant to *E. betae* infection. Similar results were obtained with other crops and pathogens that used the same technique (Awad and El-Ghonemy, 2015). Abu-Ellail and El-Mansoub (2020) also demonstrated a considerable divergence in the percent disease severity amongst the varieties. Heba cv. had the least disease severity, followed by Beta382 and Pleno cv. compared to the other treated varieties. Conversely, Oscar poly cv. had the highest percent disease severity, and a significant discrepancy ( $P < 0.05$ ) was observed among varieties for the infection score.

### **4.2 Sugar beet: yield and quality.**

The Puma cultivar resulted in the highest root yield, while the Top cultivar had the lowest yield. Among the other six cultivars

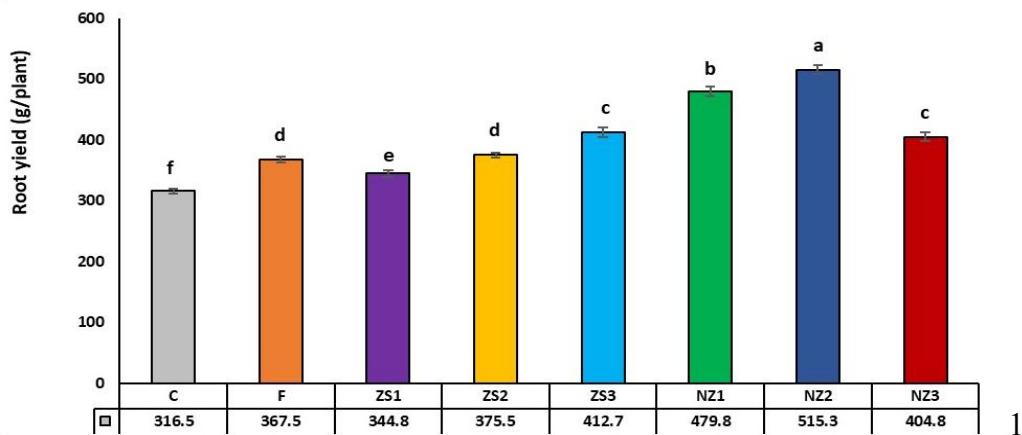


Figure 4. Effect of chemical inducers on root yield (g/plant) of sugar beet (Top cultivar) infected with powdery mildew disease under greenhouse conditions.

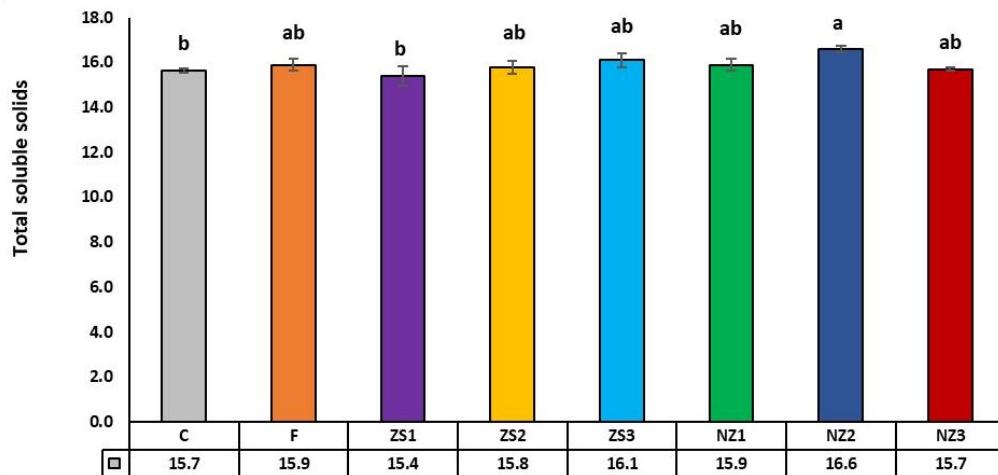


Figure 5. Effect of chemical inducers on total soluble solids (%) of sugar beet (Top cultivar) infected with powdery mildew disease under greenhouse conditions.

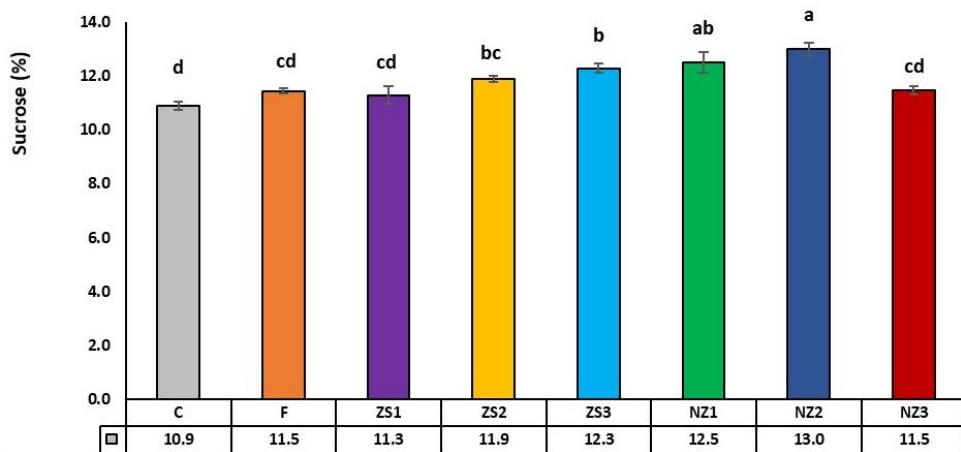


Figure 6. Effect of chemical inducers on sucrose concentration (%) of sugar beet (Top cultivar) infected with powdery mildew disease under greenhouse conditions.

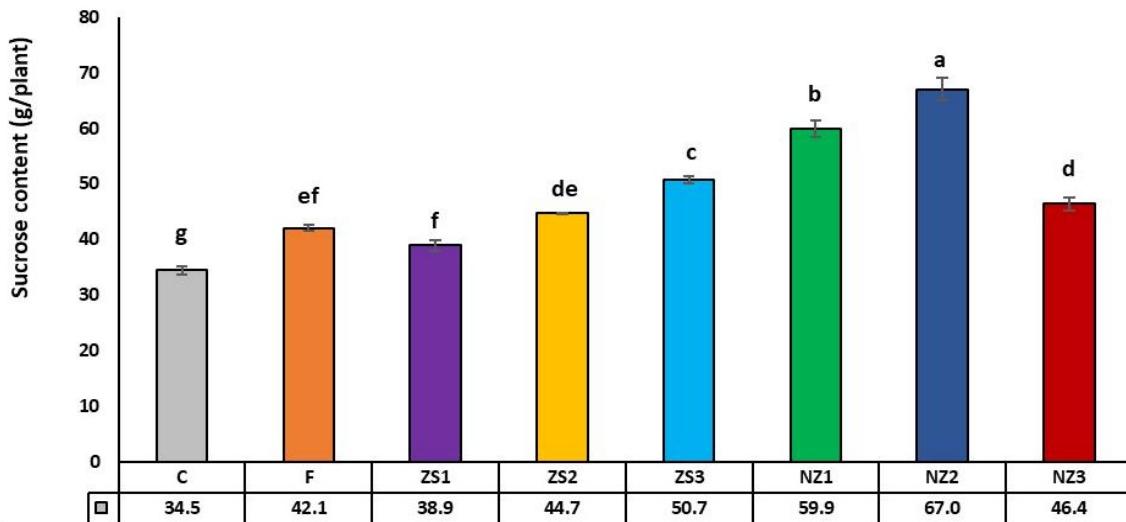


Figure 7. Effect of chemical inducers on sucrose content (g/ plant) of sugar beet (Top cultivar) infected with powdery mildew disease under greenhouse conditions.

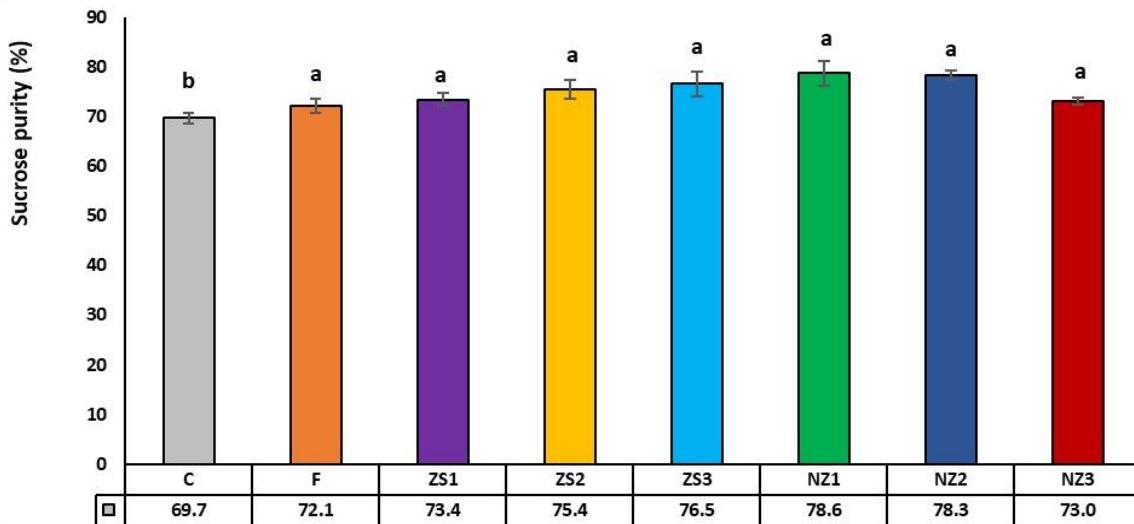


Figure 8. Effect of chemical inducers on sucrose purity (%) of sugar beet (Top cultivar) infected with powdery mildew disease under greenhouse conditions.

tested under artificial inoculation, Oscar Poly and Gazell cultivars had high yields, followed by Heba and Lola cultivars with moderate yields, and Carola and Toro with low yields, respectively. These results are consistent with the findings of El-Sayed (2015), who reported that the highest root weight was observed in the highly resistant variety Ymer, followed by

MeridiaHM, Ernestina, Puma, and Lola. Furthermore, the roots of these cultivars had the highest percentages of T.S.S., purity, and sucrose.

#### 4.3 Effect of the chemical inducers to powdery mildew on sugar beet and it's yield and quality

Constructed on the results of the first experiment, the TOP cultivar was selected as the most sensitive variety, to conducted testing some chemical inducers to induce sugar beet resistance to powdery mildew disease. The chemical inducers included nano-ZnO (NZ) at different concentrations (10, 50 and 100 mg l<sup>-1</sup>), zinc sulfate (ZS) at the same concentrations (10, 50 and 100 mg l<sup>-1</sup>), (Opera) fungicide at recommended dose (1cm), and control (untreated plants) under controlled greenhouse conditions.

Our results agreed with (Shabrawy and Abd Rabboh, 2020) Who revealed that the foliar application by micro-nutrients like copper sulfate 30ppm was the utmost treating from micro-nutrient which arrives following eminent treating tracked by zinc sulfate 30ppm, ferrous sulfate 30ppm and magnesium sulfate 30ppm were least micro-nutrient ineffecency from the other micro-nutrient. (Eliwa et al., 2018) they stated that utilizing those micro-nutrient like (zinc sulfate with concentrations 10, 20 and 40ppm) that resulted great decreasing of sugar beet powdery mildew and rising micro-nutrients concentricity raising resistance of sugar beet versus disease of powdery mildew. Micro-nutrients acted an essential role in metabolism of plant by impacting the lignin and phenolic content and stable of membrane too (Graham and Webb, 1991). Micro-nutrients could impact resistance indirect way, as in deficient plants they befit better appropriate feeding substrate.

Current study had carried out for inspect impact of some chemical molecules (elicitors) for inducing resistance of sugar beet plants against *Erisiphe betae* through greenhouse

conditions. The fungui is capable to germinatein dry-eco-climates and so infect host plants. The activation of the oxidative enzymes rises gradually following the appearance of the mycelium of *E.betae* on the foliar surfaces, therefore confirming their value at the public-defense strategy of the plant against oxidative pressure. Except for the superoxide-dismutase, in during the incubating period, there wasn't noted powerful stimulation of the examined enzymes.

#### **4.4 Effect of chemical inducers on powdery mildew disease severity (DS (%)) of sugar beet plants**

The present study demonstrated that exogenous applications of Opera, nZnO<sub>3</sub>, 2 with its concentrations (100 and 50 ppm) and SZ3, 2 (100 and 50 ppm), as foliar spray can induce resistance of plants of sugar beet against the disease of powdery mildew happened by *E.betae*. Former outcomes showed the probable importance of those elicitors in mitigating the deleterious effects caused by biotic stress on plants (Elsheery, 2020), which play a significant part in plant defense responses for pathogen attack.

These results were so similar to (Derbalah et al., 2013), who indicated that the disease severity of Cercospora leaf blight of sugar beet were reduced within foliar application of ZnONPs at 500 µ g/mL. (Ahmad, L. and Siddiqui, 2021) mentioned that zinc (ZnO NPs) oxide nanopartciles formed agent of stabilizing for membranes of the cell and might guard plants against pathogens too. ZnO NPs have an important potency to consolidate agriculture due in their fabulous physical, optical and also antimicrobial features. Additionally, these NPs shape an

antimicrobial efficacious agent against plants' pathogens (Sabir et al., 2014).

Results indicated that all chemical inducers, particularly Opera, nZnO<sub>3</sub>, 2 (100 and 50 ppm) and SZ3, 2 (100 and 50) followed by nZnO1 (10ppm) and SZ1 (10ppm), significantly reduced percent disease severity [DS (%)] of the whole plant leaves from 13 to 45 DAI (4 days interval) as well as the percent disease severity (%) of the upper non-treated leaves at 45 DAI of sugar beet plants inoculated with *E.betae* compared to infected control. These results proved that, there are synergistic effects among tested inducers (Opera and nZnO<sub>3</sub> (100 ppm)) for inducing resistance of sugar beet plants against powdery mildew fungus (*E.betae*).

Present study concluded that nZnO and SZ acted in synergistically for decreasing the unwholesome impacts of powdery mildew disease happened by *E.betae* in plants of sugar beet cross induced resistance against this pathogen. Furthermore, the physiological role of these elicitors for inducing resistance against powdery mildew disease could be related to increasing concentrations of photosynthetic pigments and total soluble sugars.

#### 4.5 Photosynthetic pigments

Biotrophic-fungal pathogens infect plants, involving of powdery mildew disease, showed decreasing average photosynthesis (Prokopová et al., 2010). Current results revealed that, concentration of photosynthetic pigments (chlorophylla,b,A/B and total cholorophyll) in control plants decreased significantly in response to inoculation with *E.betae*. These results are according to

Radwan et al. (2007) who mentioned that, the photosynthetic pigments of sugar beet leaves were significantly declined responding to (ZYMV) zucchini yellow mosaic virus. In addition, Rathod and Chatrabhuji, 2010 who reported that the concentration of total chlorophyll decreased in mustard (*Brassica juncea*) leaves are responding for infection by powdery mildew (*Erysiphe polygoni*) compared with healthy leaves. Furthermore, Kobeasy et al. (2011) proved that healthy peanut plants recorded higher chlorophyll and carotenoids concentrations than those in plants infected with mottle virus. Moreover, they found that chlorophyll concentration in barley plants was deceased significantly after 4 days of infection with powdery mildew disease.

The reduction of photosynthetic pigments due to powdery mildew infection may be accomplished with inhibiting of electron transport, alternations in the ultrastructure of chloroplasts and also reduction of enzyme activity (Percival and Fraser, 2002). Moreover, it might dampen photosynthetic operations with lower supplying of light-energy because leaf's surface covered with mycelium and also damping of flux CO<sub>2</sub> because of closing the stomata. Before inoculation, i.e. in healthy plants, Opera fungicide, nZnO<sub>3</sub> (100 ppm), nZnO<sub>2</sub> (50 ppm) and their combinations significantly raised concentricities of chlorophyll (a, b and A/B) and total chlorophyll to compare with control. Maximum concentrations of photosynthetic pigments were recorded by Opera and nZnO<sub>3</sub> (100 ppm) for chlorophyll as well as nZnO<sub>2</sub> (50 ppm) for total chlorophyll.

Biotrophic fungal pathogens, including powdery mildew that infected Plants, exhibited decreased photosynthesis's rate (Prokopová et al., 2010). The present study showed that the foliar spray with nano-ZnO and ZnSO<sub>4</sub> at divergence concentricities (10, 50 and 100 mg l<sup>-1</sup>) prior 72 h of artificial inoculation with *E. betae* gave dissimilar responses. Chl a and b concentration significantly decreased after inoculation (AI) compared with before inoculation (BI). This result agreed with (El-Fahar and Abo El-Magd, 2008) who reported that chlorophyll content was highly affected because powdery mildew infection decreased photosynthetic activity. The alterations in the concentration of chlorophyll could be result from influence of the pathogen' impacts. Decreasing in content of chlorophyll were resulted from chloplast strucral modulation by fungi like expansion of entire chloroplast, segregation the accumulating of grana from starch' granules, that own directly impact on photosynthetic capability of chloroplast. (Laware and Raskar, 2014) demonstrated that out of the four different concentrations of ZnO NPs ( $\approx$ 18 nm) (10, 20, 30 and 40  $\mu$ g/ml), the optimum concentrations, 20 and 30  $\mu$ g/ml were found to be beneficial at the highest to seed germination, plant growth, flowering by reducing flowering period and in the production of healthy seeds in plants. Ismail and Abd El-Gawad, (2021) showed that ZnONP penetration may cause greater internal ZnO-induced oxidative damage than outside the cells. (Radwan et al., 2007) In addition, (Rathod and Chatrabhuji, 2010) reported that the concentration of total chlorophyll decreased in mustard (*Brassica juncea*) leaves in response to infection with

powdery mildew (*E. polygoni*) compared to healthy leaves. Furthermore, Kobeasy et al. (2011) proved that healthy peanut plants recorded higher chlorophyll and carotenoids concentrations than those in plants infected with mottle virus. Hafez et al. (2018) also mentioned that the reduction of photosynthetic pigments due to powdery mildew infection may be associated with inhibition of electron transport, alternations in the chloroplasts ultrastructure and reduction of enzyme activity (Percival and Fraser, 2002). Moreover, powdery mildew can inhibit the photosynthetic processes by lower supply of light energy due to covering of the leaf surface by mycelium and inhibition of CO<sub>2</sub> influx due to stomata closure. It is better to mention that, the nano zinc-oxide and the fungicide Opera improved photosynthetic pigments in sugar beet plants either prior or post inoculation compared to infected control. Their impact may due to mechanism of Opera and nZnO<sub>3</sub>. However, plants could afford infection caused by herbivores or pathogens by raising the concentricity of chlorophyll in their leaves (Lattanzio et al., 2006).

#### 4.6 Total soluble sugars and sugar beet weight

Whole tested treatments greatly raised root yield compared with control. A negative connection was among TSS and disease severity. These are agreed with Xiao et al., 2022 who mentioned that pathogens might absorb nutrients from leaves, in especially glucose, as a main carbon energy source. Infection impacts the source sink distributing of plants that resulted in a boosted sugar uptake capacity of the leaves and the activity of sucrose degradation enzymes. Wang et al., 2014 stated that the decreasing of sugar

content referred the lowering of CO<sub>2</sub> carboxylation and formation of starch. Diversion among sugar and starch is another indicative method in responding to plant stress. Kontradowitz and Verreet, 2010 registered that powdery mildew in sugar beet reduced root yield, yield quality and controlling the disease resulting a raise substantial of root yield and yield quality. The role of Zn in assisting the utilization of nitrogen and phosphorus in plants could be responsible for the augmentation in top and root fresh weights gained by Zn application (Enan, 2004).

In this concern, nZnO<sub>3</sub>, 2 (100 and 50 ppm) registered greatly the utmost increasing in sugar beet contents of both sucrose percent and T.S.S tracked by fungicide Opera. In another way, nZnO<sub>1</sub> (10 ppm) and SZ1 (10 ppm) registered the lowest greatly impacts on both sucrose percent and T.S.S in sugar beet. Opera and nZnO<sub>3</sub> (100 ppm) treatments achieved the greatest increasing in the percentage of purity in sugar beet among the investigated treatments while nZO1 (10 ppm) and SZ1 (10 ppm) treatment registered the minimal increasing in purity percentage and, such increase seemed to be insignificantly more raising than the infected control. Outcomes were agreeing with previous outcomes which recorded by Kontradowitz and Verreet, (2010), who stated that sugar beet powdery mildew disease reduced yield quality and root yield.

The concentricity of total soluble sugars (TSS) raised greatly post inoculation compared with prior inoculation either in control or treated plants. Agreeing with this result, also wheat plants subjected to powdery

mildew disease showed accumulation in their contents of soluble carbohydrates, which may be due to stimulated activity invertase at leaves mildewed.

According to Prokopová et al. (2010), through powdery mildew infection that caused with raising invertase activity in cell-wall, had been showed the down-regulation of photosynthesis, that led to hexose sugars accumulating. In the present work, Opera and nZnO<sub>3</sub> (100 ppm) attained the highest concentrations of TSS either in healthy or in inoculated sugar beet plants. It is worthy to denote that, increasing TSS concentration significantly contributed to induce resistance of plants against biotical stress which happened by pathogen or sherbivores. Moreover, Conrath, (2009) reported that elevated levels of soluble sugars are considered one type of induced resistance in plants that called "high-sugar resistance", where cumulating of sugars in plants could lead to activation of many-diverse pathogenesis-related genes.

## 5. Conclusion

We conclude from our results that Zn NPs at concentrations of 100 ppm significantly increased levels of chlorophyll, total soluble sugars, endogenous H<sub>2</sub>O<sub>2</sub>, and activities of peroxidase and polyphenol oxidase, subsequently reducing the severity of powdery mildew disease. The physiological role of Zn NPs in inducing resistance against the pathogen is attributed to the production of reactive oxygen species scavenge such as phenolic compounds and POD. Overall, the findings of this study suggest ZnO nanoparticles can be used as a foliar spray to

reduce the harmful impacts of biotic stress caused by *E. betae* in sugar beet plants by inducing resistance to the pathogen, providing a potential solution for sustainable crop management and environment.

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### Availability of Data and Materials

Data will be available on demand from the corresponding authors.

### Authors Contributions

H.H.A., and A.B.E., convinced the main idea. H.F.M., and N.I.E., designed the experiment, wrote the manuscript. All authors helped in data collection, data analysis and revising manuscript. The Author (s) read and approved the final manuscript.

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